HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE

PUBLISHED BY THE

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

VOL. 2 APRIL, 1927 NO. 15

VARIATION IN THE REACTIONS OBTAINED IN REPEATED AGGLUTINATION TESTS OF THE SAME FOWLS WITH *BACTERIUM PULLORUM* ANTIGEN

J. R. BEACH[1]

INTRODUCTION

The studies by Rettger[1, 2, 4] and Rettger and Harvey,[3] reported in four papers published between 1900 and 1909, definitely established the disease of young chicks commonly known as "white diarrhea" to be a specific infectious disease, the causative organism of which was designated *Bacterium pullorum*. Further studies by Rettger and his associates were reported in 1909,[5] 1911[6] 1912,[7] and 1914.[8, 9] They determined that apparently healthy adult fowls may be carriers of *Bact. pullorum*. The infection in hens usually becomes localized in the ovaries and is eliminated in the eggs. When such eggs are used for hatching, the infection is transmitted to chicks. This is considered the usual source of *Bact. pullorum* infection in chicks. Jones[10, 11] in 1910 and 1911 and Gage[12] in 1911 published the results of investigations which confirmed the findings of Rettger and his associates.

The most important problem in the prevention of the disease in chicks, therefore, became the detection and elimination of infected breeding stock. In 1913 Jones[13] demonstrated that the agglutination test was of value for this purpose. His findings were confirmed by others and the testing of breeding flocks by this method has been practiced extensively for several years.

[1] Assistant Professor of Veterinary Science and Associate Veterinarian in the Experiment Station.

All who have made careful study of the agglutination test for detecting carriers of *Bact. pullorum* recognize that repeated tests are necessary for the elimination of all infected fowls in a flock. The failure of a single test to detect all of the infected fowls is commonly considered to be due either to certain birds having acquired infection too recently for the production of sufficient agglutinins in their blood serum to cause an agglutination reaction or to certain birds becoming infected after the test, either from association with the infected birds or from contaminated litter and soil. It is commonly considered, however, that after agglutinins have become sufficiently abundant to cause a reaction, they will remain so as long as the fowl continues to harbor *Bact. pullorum.*

The workers in the laboratories of the Division of Veterinary Science, University of California had no reason to doubt that fowls with well-established infection with *Bact. pullorum* would uniformly react to the agglutination test until, in the routine testing that was being carried on, it became necessary to make tests of two lots of blood samples from the same birds at an interval of twenty-one days. On August 25, 1925, a test was made on a lot of 574 blood samples. Another lot of blood samples from this flock was received on September 15, 1925, and included duplicates of 388 samples that had been tested twenty-one days previously. The comparative results of the tests of the two samples of blood from 388 fowls were as follows:

Six fowls reacted to both tests.

Twelve fowls that gave a positive reaction to the first test failed to react to the second test.

Three fowls that were negative at the first test reacted to the second test.

Such discrepancies in the results of the two tests could not be ascribed to differences between the antigens or methods because these were the same for both tests. They could hardly be considered as due to certain fowls having become free from and others having acquired infection between the two tests because of the short interval between them. Therefore, it seemed probable that all of the fowls that had reacted to either one or both tests were infected at the time each test was made but certain of them had failed to react to one test. If this assumption is correct, it would indicate that fowls may not constantly give a positive reaction to the agglutination test while they are carriers of *Bact. pullorum.* It was to obtain information on this point that the studies herein reported were undertaken.

Other points regarding which it was thought information might be obtained were:

First, the correlation between intensity of egg production, age of the birds or season of the year and any variability in the reactions to repeated agglutination tests of the same individuals that is found to occur;

Second, the accuracy with which the results of an agglutination test may be interpreted to indicate the presence or absence of *Bact. pullorum* infection;

Third, the rapidity with which *Bact. pullorum* infection may spread among non-infected adult females from association with infected adult females.

Plan of the Experiment

In December, 1925, 200 White Leghorn pullets, from 8 to 9 months old, were obtained from a flock in which *Bact. pullorum* infection was known to exist. They were immediately leg-banded and subjected to an agglutination test. Seventy of the birds gave a positive reaction in at least one dilution and 130 failed to react. The seventy reactors and eighty of the non-reactors were placed together in one house and designated group 1 and group 2, respectively. The remaining fifty non-reactors were placed in a separate house and designated group 3. Outside runs were not provided. An agglutination test of the blood serum of all birds was made once each month. This procedure is to continue for at least two years, but this report is concerned only with the first twelve months.

A careful search for *Bact. pullorum* was made in all birds that died from any cause, except certain ones otherwise accounted for.

The antigen was prepared from a single strain of *Bact. pullorum* of known good agglutinability that had been isolated from a chick. Cultures were incubated on agar for 48 hours and the growth washed off with sterile, physiological salt solution containing 0.5 per cent phenol. Cultural and microscopic tests were made of each lot of antigen to insure freedom from contamination. The antigen in concentrated form was stored in an ice box. It was diluted with sufficient phenolized saline to give a reading of 3.5 cm. with a Gates' nephelometer at the time the tests were made.

In all of the tests, four dilutions of serum and antigen were used, i.e., 1–25, 1–50, 1–100 and 1–200. The tubes were incubated for 24 hours and kept at room temperature 24 hours longer. Readings were made after 24 and 48 hours.

Summary and Discussion of the Results of the Agglutination Tests of the Fowls in Group 1

This group consisted of seventy birds that gave a positive reaction to the first agglutination test. However, there was a marked variation in the number of these birds that reacted at each of the eleven subsequent monthly tests. A summary of the results of the tests and the average egg production during each month is given in table 1. The gradual decrease in the number of birds in the group is due to deaths that occurred.

TABLE 1

Summary of the Results of 12 Agglutination Tests and Egg Production of Group 1

Month	Number of birds in group	Number of reactors	Per cent reactors	Per cent egg production for the month
December	70	70	100.0	0.
January	70	38	54.2	1.3
February	68	39	57.3	2.9
March	60	44	73.3	27.2
April	59	29	49.1	39.5
May	58	26	44.8	45.7
June	56	23	41.0	41.4
July	54	31	57.3	28.2
August	53	26	49.0	21.7
September	51	25	49.0	21.9
October	51	22	43.1	12.2
November	50	19	38.0	3.6

In table 1, it is seen that at none of the tests after the first were reactions obtained from all of the birds that reacted to the first test. The nearest approach to this was in March, when 73.3 per cent of the birds reacted. In January, February and July, positive reactions were obtained from 54.2 per cent, 57.3 per cent and 57.3 per cent of the birds, respectively. Less than half of the birds gave a positive reaction at each of the seven other tests, the percentage ranging from 49 in April, August and September down to 38 in November. Table 1 also clearly shows that the variation in the number of birds that reacted at the different tests was not correlated to that of egg production.

The progressive decrease in the number of reactors to each of the tests after July suggests that the decrease may be in correlation with

the increasing age of the birds. However, a similar decline in number of reactors occurred between March and June, but was followed by an increase in the number of reactors to the test in July.

The difference in the number of the birds of group 1 that reacted to each of the twelve tests was not merely a progressive decrease due to certain of the birds ceasing to react, but was also due to a fluctuation between positive and negative of the reactions which some individual birds gave to the different agglutination tests. This is shown by the increase in the number of positive reactions obtained in March over that obtained in January or February and in the number in July over that obtained in April, May or June. It is more clearly brought out, however, by the following detailed summary of the reactions to the agglutination test of the fifty birds that lived during the entire year and were tested twelve times.

A general summary of the number of the fifty birds that reacted to each of the twelve tests is given in table 2.

TABLE 2

SUMMARY OF RESULTS OF AGGLUTINATION TESTS OF 50 BIRDS OF GROUP 1 THAT WERE TESTED TWELVE TIMES

Number of test	Month	Number of reactors	Per cent reactors
1	December	50	100.0
2	January	31	62.0
3	February	28	56.0
4	March	36	72.0
5	April	23	46.0
6	May	21	42.0
7	June	19	38.0
8	July	27	54.0
9	August	24	48.0
10	September	24	48.0
11	October	21	42.0
12	November	19	38.0

In table 2, it is seen that the variation in the percentage of the fifty birds that gave positive reactions to the different agglutination tests closely follows that shown in table 1 for the whole of group 1.

Of the fifty birds that were tested twelve times

10, or 20 per cent gave a positive reaction to all 12 tests.
4, or 8 per cent gave a positive reaction to 11 tests.
4, or 8 per cent gave a positive reaction to 10 tests.
2, or 4 per cent gave a positive reaction to 9 tests.

1, or 2 per cent gave a positive reaction to 8 tests.
3, or 6 per cent gave a positive reaction to 7 tests.
1, or 2 per cent gave a positive reaction to 6 tests.
4, or 8 per cent gave a positive reaction to 5 tests.
4, or 8 per cent gave a positive reaction to 4 tests.
5, or 10 per cent gave a positive rtaction to 3 tests.
4, or 8 per cent gave a positive reaction to 2 tests.
8, or 16 per cent did not react after the first test.

The distribution of the positive and negative reactions to the agglutination tests of the forty birds that did not give a positive reaction to all of the twelve tests is given in table 3.

A study of table 3 shows that the positive reactions of twenty-six of the thirty-two fowls that reacted to from two to eleven tests were interspersed with negative reactions to one or more consecutive tests. The most commonly occurring irregularity of this nature was one negative reaction between two positive reactions. This occurred in nineteen instances. Negative reactions to two consecutive tests between positive tests occurred in seven instances; to three consecutive tests in three instances; to four consecutive tests in three instances; to five consecutive tests in three instances; and to seven consecutive tests in one instance.

Table 3 also shows that certain of the birds, after giving positive reactions either consistently or irregularly to one or more tests, did not react to any subsequent test. The number of such birds and the last test to which a positive reaction was obtained is as follows:

8 birds did not react after the first test.
2 birds did not react after the third test.
3 birds did not react after the fourth test.
3 birds did not react after the fifth test.
3 birds did not react after the eighth test.
1 bird did not react after the ninth test.
5 birds did not react after the tenth test.
4 birds did not react after the eleventh test.

The disappearance of agglutinins from the blood serum of the sixteen birds that failed to react after the first, third, fourth or fifth test is possibly due to the birds having become free from *Bact. pullorum* infection. These birds cannot with certainty be regarded as free from infection, however, because, as will be shown later, *Bact. pullorum* was isolated from the ovaries of six birds of group 1 that died after having failed to react to from one to three agglutination tests next preceding their deaths.

TABLE 3

THE DISTRIBUTION OF THE POSITIVE AND NEGATIVE REACTIONS OF 40 BIRDS OF GROUP 1 THAT DID NOT REACT TO ALL OF THE TWELVE AGGLUTINATION TESTS

Number of positive reactions	Total number of birds	Number of birds that gave the same reaction to each test	Tests at which a positive reaction occurred	Tests at which a negative reaction occurred
11	4	1	First and second; fourth to twelfth	Third
		1	First to sixth; eighth to twelfth	Seventh
		2	First to eleventh	Twelfth
10	4	1	First to fifth; seventh to ninth; eleventh and twelfth	Sixth and tenth
		1	First to tenth	Eleventh and twelfth
		1	First to fourth; sixth; eighth to twelfth	Fifth and seventh
		1	First to third; fifth to eleventh	Fourth and twelfth
9	2	1	First to fourth; sixth to tenth	Fifth, eleventh and twelfth
		1	First and second; sixth to twelfth	Third, fourth and fifth
8	1	1	First; third to fifth; eighth and ninth; eleventh and twelfth	Second, sixth, seventh, tenth
7	3	1	First to fourth; ninth and tenth; twelfth	Fifth to eighth; eleventh
		1	First; seventh to twelfth	Second to sixth
		1	First to sixth; eleventh	Seventh to tenth; twelfth
6	1	1	First and second; fourth to sixth; eighth	Third; seventh; ninth to twelfth
5	4	1	First to fifth	Sixth to twelfth
		1	First and second; fourth and fifth; eighth	Third; sixth and seventh; ninth to twelfth
		1	First to fourth; twelfth	Fifth to eleventh
		1	First to third; eighth and ninth	Fourth to seventh; tenth to twelfth

TABLE 3—(*Continued*)

Number of positive reactions	Total number of birds	Number of birds that gave the same reaction to each test	Tests at which a positive reaction occurred	Tests at which a negative reaction occurred
4	4	1	First and second; fourth and fifth	Third; sixth to twelfth
		1	First; fourth; eighth; tenth	Second and third; fifth to seventh; ninth; eleventh and twelfth
		1	First to fourth	Fifth to twelfth
		1	First, third and fourth; tenth	Second; fifth to ninth; eleventh and twelfth
3	5	1	First and second; fourth	Third; fifth to twelfth
		1	First; fourth; tenth	Second and third; fifth to ninth; eleventh and twelfth
		1	First; fourth; eighth	Second and third; fifth to seventh; ninth to twelfth
		1	First; fourth and fifth	Second and third; sixth to twelfth
		1	First, second and third	Fourth to twelfth
2	4	3	First and fourth	Second and third; fifth to twelfth
		1	First and third	Second; fourth to twelfth
1	8	8	First	Second to twelfth

Any or all of the birds that gave positive reactions up to the eighth or subsequent tests can reasonably be expected to again react since the number of tests to which these birds have given a negative reaction is no greater than the number of consecutive negative reactions that occurred between the positive reactions of some of the birds that reacted irregularly to the tests.

The variation in the number of birds of group 1 that reacted to each of the twelve agglutination tests made at intervals of approximately one month is, therefore, manifested in two ways: first, by fluctuation between positive and negative of the reactions of some individuals to the different tests, and, second, by certain of the birds, after giving a positive reaction to one or more tests, ceasing to react to all subsequent tests.

Studies by Beach, Halpin and Lampman[14] that were carried on at the same time as those herein reported showed similar variation in the reactions to the agglutination test exhibited by a flock of hens that was tested twelve times in thirteen months.

Summary of Results of the Agglutination Tests and Postmortem Examination of the Fowls that Died in Group 1

The mortality in group 1 during the year was twenty fowls. Two were not examined. The remaining eighteen were carefully examined for the presence of gross ovarian or other lesions suggestive of *Bact. pullorum* infection. A bacteriologic examination, particularly for the purpose of determining the presence of *Bact. pullorum,* was made of the ovaries and yolks of these birds. The results are given in table 4:

TABLE 4

Results of Agglutination Tests and Postmortem Examination of Twenty Fowls that Died in Group 1

Number of agglutination tests	Tests giving positive reaction	Tests giving negative reaction	Condition of ovary	Ovarian lesions found	Results of bacteriologic examination of ovaries
2	First	Second	Active	None	Negative
3	All	None	Dormant	Abnormal yolks	*Bact. pullorum* isolated
3	First	Second and third	Dormant	Congestion. No abnormal yolks	*Bact. pullorum* isolated
4	First and second	Third and fourth	Dormant	Abnormal yolks	*Bact. pullorum* isolated
7	First, third and fourth	Second, fifth, sixth, seventh	Not examined		
8	All	None	Active	Abnormal yolks	*Bact. pullorum* isolated
10	All	None	Dormant	Abnormal yolks	*Bact. pullorum* isolated
11	All	None	Dormant	Abnormal yolks	*Bact. pullorum* isolated
3	First	Second and third	Dormant	Abnormal yolks	*Bact. pullorum* isolated
2	First	Second	Dormant	None	*Bact. pullorum* isolated
3	First and third	Second	Dormant	Abnormal yolks	*Bact. pullorum* isolated
6	First to fifth	Sixth	Dormant	Abnormal yolks	*Bact. pullorum* isolated
3	First and third	Second	Dormant	Abnormal yolks	*Bact. pullorum* isolated
5	First, third, fourth	Second, fifth	Active	Abnormal yolks	Negative
9	First, third, fourth, sixth, eighth	Second, fifth, seventh, ninth	Active	Abnormal yolks	*Bact. pullorum* isolated
3	First	Second, third	Dormant	Abnormal yolks	*Bact. pullorum* isolated
6	First	Second to sixth	Not examined		
8	All	None	Dormant	Abnormal yolks	*Bact. pullorum* isolated
3	First	Second, third	Dormant	Abnormal yolks	*Bact. pullorum* isolated
4	First	Second, third, fourth	Active	Abnormal yolks	*Bact. pullorum* isolated

A study of the summarized data concerning the twenty dead fowls from group 1, as given in table 4, shows the following:

No examination was made of two fowls.

Bact. pullorum was isolated from the ovaries of sixteen of the eighteen fowls examined. Gross ovarian lesions, in the form of abnormal yolks, were present in fourteen of these. Congestion of the ovary was the abnormality found in one. In the one remaining fowl from which *Bact. pullorum* was isolated, no ovarian abnormality nor other lesion suggestive of infection with *Bact. pullorum* was observed. Five of the sixteen fowls that yielded *Bact. pullorum* at the bacteriologic examination reacted to all agglutination tests before their death. Three failed to react to all tests but did to the one next preceding their death. Two did not react to the last agglutination test, five did not react to the last two tests, and one did not react to the last three tests, preceding their death. This definitely shows fowls with well-established ovarian infection with *Bact. pullorum* may not always have sufficient agglutinins in their blood serum to cause a reaction to the agglutination tests.

The ovary of one of the two reacting fowls from which *Bact. pullorum* was not isolated was normal in appearance. The ovary of the other bird contained abnormal yolks. The negative results of the bacteriologic examination of these two birds cannot be considered as positive evidence that they were not carriers of *Bact. pullorum*. The organism may have been present in them even though it was not recovered in cultures.

The comparative results of the agglutination tests and bacteriologic examination of the eighteen dead fowls indicate that a high percentage of fowls that give a positive reaction to an agglutination test for the detection of *Bact. pullorum* infection are carriers of that organism. This is true even though such fowls fail to react to agglutination tests made one to three months later. As previously stated, the term "positive reaction" in this paper is applied to partial or complete clearing of any one or all of the four serum-antigen dilutions, 1–25, 1–50, 1–100 and 1–200.

Summary of Results of the Agglutination Tests of the Fowls in Group 2

Group 2 consisted of eighty of the fowls that did not react to the first agglutination test and that were confined in the same pen with group 1, the fowls that reacted to the first test.

Twelve of these fowls reacted to some of the subsequent agglutination tests. The tests to which these birds gave positive reactions were as follows:

One fowl gave a positive reaction to the second, third, fourth and fifth tests, and a negative reaction to the sixth test. This bird afterwards disappeared from the pen and no further data concerning it was obtained.

One fowl gave a positive reaction to the fourth, fifth and eighth to twelfth tests.

One fowl gave a positive reaction to the fourth, fifth, eighth and ninth tests.

One fowl gave a positive reaction to the fourth and eighth tests.

Five fowls gave a positive reaction to the fourth test only. The reactions of two of these consisted only of a partial clearing of the lowest of the four serum-antigen dilutions. Since these two birds gave only a slight reaction to the one test and no reaction to the other tests, it is perhaps incorrect to classify them as reactors.

One fowl gave a positive reaction to the eighth and ninth tests.

One fowl gave a positive reaction to the tenth test.

One fowl gave a positive reaction to the eleventh and twelfth tests.

With the exception of the one that disappeared from the pen, all of the fowls that became reactors to the agglutination test are still living. No opportunity for postmortem and bacteriologic examination of any of them has, therefore, been afforded.

Nine of the fowls were negative to from one to three agglutination tests before they gave a positive reaction. This number of negative reactions is no greater than the number of consecutive negative reactions that occurred between the positive reactions of some of the birds of group 1. Therefore, it seems just as probable that the positive reactions of these birds resulted from *Bact. pullorum* infection which they were harboring when the experiment started as from infection which they acquired from association with the infected birds.

The other three birds that became reactors did not react until the eighth, tenth and eleventh tests, respectively. It does not seem improbable, therefore, that the positive reaction to the agglutination test of these birds was due to infection with *Bact. pullorum* acquired after the experiment started. This indicates that transmission of *Bact. pullorum* infection among adult fowls by association of infected and non-infected may be an important factor in increasing the extent of the infection in breeding flocks.

Results of Postmortem Examinations of the Fowls in Group 2 that Died

Twenty-four hens died during the year. None of these had given a positive reaction to an agglutination test. Three of the dead were not examined. Twenty-one were given a careful postmortem examination for gross lesions, particularly of the ovary, that might be suggestive of *Bact. pullorum* infection. Cultures were made from the ovaries of these birds.

The bacteriologic examination of eighteen birds gave negative results. The ovaries of eleven of these birds were normal in appearance; a small cyst was attached to the ovaries of two; the ovaries of two birds were congested; and a few small abnormal yolks were present in three birds.

Bact. pullorum was isolated from the ovaries of the remaining three of the twenty-one birds examined. Abnormal yolks were present in all three birds. Two of these had been negative to three agglutination tests and one to five tests. This is additional evidence that fowls with infection of the ovaries with *Bact. pullorum* may fail to react to the agglutination test.

Summary of the Results Obtained with Group 3

This group consisted of fifty fowls that did not react to the first test and were kept separated from all other fowls during the year.

Forty-nine of the birds did not react to any of the agglutination tests during the year. Twelve of these birds died during the year. The deaths occurred after they had been tested from two to ten times. Examination was not made in the case of six of these. Postmortem examination of the remaining six showed the ovaries to be normal in appearance with the exception of a pea-sized tumor-like mass of tissue attached to the ovary of one bird. The bacteriologic examination of these six fowls gave negative results.

One fowl reacted to the fifth and sixth tests. This fowl died soon after the sixth test. The ovary contained several large abnormal yolks from which *Bact. pullorum* was isolated. It seems unlikely that this bird became infected from association with the other birds of group 3 since the experiment started as the results of the agglutination tests and the postmortem examination of those that died indicate that none of the other birds were infected. Furthermore, the lesions, found in

this fowl seemed too extensive to have resulted from recently acquired infection. It appears probable, therefore, that although this fowl did not give a positive reaction until the fifth month of the experiment, it was harboring infection when the experiment started.

Variation in the Degree of the Positive Reactions to the Agglutination Tests

In preceding pages, it has been pointed out that reactions to the agglutination tests of many of the birds fluctuated between positive and negative. A reaction was considered to be positive when there was entire or partial clearing of any one of the four serum-antigen dilutions. It was found that a great variation existed between the degree of the positive reactions of the birds that reacted more than once. In fact, the blood serum of no bird that reacted to more than one test caused the same degree of agglutination in all of the tests in which a positive reaction was secured. The total number of positive reactions obtained was 418. The variation in the degree of reactions is summarized in table 5.

TABLE 5

Variation in the Degree of the 418 Positive Reactions to the Agglutination Test

Dilutions in which agglutination occurred*	Number of reactions	Per cent of total reactions
Partial in 1–25 dilution. No agglutination in others	26	6.1
Complete in 1–25 dilution. No agglutination in others	72	17.2
Partial or complete in 1–25 and 1–50 dilutions. No agglutination in others	133	29.4
Partial or complete in 1–25, 1–50 and 1–100 dilutions. No agglutination in others	71	16.9
Partial or complete in 1–25, 1–50, 1–100 and 1–200 dilutions	116	25.3

* In every instance, there was agglutination in all dilutions below the highest dilution in which agglutination occurred.

It is shown by table 5 that fewer positive reactions would have been obtained if the lowest serum-antigen dilution had been higher than 1–25. If the lowest dilution had been 1–50 there would have been 98 or 23.4 per cent less positive reactions, if the lowest dilution had been 1–100, there would have been 231 or 55.2 per cent less positive reactions.

Such variation in the results of the agglutination tests gives rise to the question of whether an agglutination in low dilution only can be interpreted as indicating infection with *Bact. pullorum*. Some information on this point is furnished by the positive reactions to the agglutination test of seventeen reacting fowls which died and from which *Bact. pullorum* was isolated. Fifty-nine positive reactions were obtained from these seventeen birds. Table 6 gives a summary of the variation in the degree of the reactions.

TABLE 6

VARIATION IN THE DEGREE OF 59 POSITIVE REACTIONS TO THE AGGLUTINATION TESTS OF 17 FOWLS THAT DIED AND FROM WHICH *Bact. pullorum* WAS ISOLATED

Dilutions in which agglutination occurred	Number of reactions	Per cent of total reactions
Partial in 1–25 dilution. No agglutination in others	3	5.0
Complete in 1–25 dilution. No agglutination in others	13	22.0
Partial or complete in 1–25 and 1–50 dilutions. No agglutination in others	20	33.8
Partial or complete in 1–25, 1–50 and 1–100 dilutions. No agglutination in others	11	18.6
Partial or complete in 1–25, 1–50, 1–100 and 1–200 dilutions	12	20.3

By comparing table 6 with table 5, it is seen that the variation in the degree of the positive reaction to the agglutination tests of the seventeen fowls that were known to harbor *Bact. pullorum* closely follows the variation in the degree of the positive reactions of all of the birds. The fact that in more than half of the positive reactions of these seventeen known infected fowls agglutination was obtained only in one or both of the 1–25 and 1–50 dilutions indicates that any fowl that gives a positive agglutination reaction in these dilutions but not in higher dilutions may be a carrier of *Bact. pullorum*. It would, therefore, be expected that any agglutination test procedure for the detection of carriers of *Bact. pullorum* should include a serum-antigen dilution as low as 1–25.

Conclusions

This paper presents the results of the first twelve of a series of at least twenty-four monthly agglutination tests of the same fowls for the detection of *Bact. pullorum* infection, together with the results of the bacteriologic examinations of the fowls that have died during the twelve month period. Complete interpretation of the results of these tests cannot be made until the experiment is terminated and a postmortem and bacteriologic examination is made of all of the fowls. The information obtained from the results of the first year of the experiment, however, would seem to warrant the following conclusions:

Adult fowls with well-established ovarian infection with *Bact. pullorum* may not always react to an agglutination test. This factor seriously affects the dependability of the agglutination test as a means of detecting *Bact. pullorum* carriers and therefore detracts from the practical value of the tests as a means for the complete eradication of the infection from a breeding flock.

A fowl that has reacted to an agglutination test may not react to subsequent tests even though it is still infected with *Bact. pullorum*. Therefore, a fowl that has once reacted to a test cannot be considered as free from the infection if it fails to react to tests that are made subsequently.

A positive reaction to the agglutination test may be considered as a highly accurate indication of *Bact. pullorum* infection. A negative reaction to a test, however, appears to less accurately indicate freedom from *Bact. pullorum* infection, either recently acquired or of long standing.

In an agglutination test procedure with an antigen of equal or greater density than that used in these studies, a serum-antigen dilution at least as low as 1–25 should be included. Clearing of the 1–25 dilution alone or accompanied by clearing of one or more higher dilutions of the same serum can be interpreted as a positive reaction.

No information regarding the interpretation of proagglutination or paradoxical reactions was obtained in these studies since this phenomenon was not encountered.

LITERATURE CITED

[1] Rettger, L. F.
1900. Fatal septicemia among young chicks. New York Med. Jour. **71**: 803–805.

[2] ———
1901. Fatal septicemia in young chicks. New York Med. Jour. **73**: 267–268.

[3] Rettger, L. F., and S. C. Harvey
1908. Fatal septicemia in young chickens or "white diarrhea." Jour. Med. Res. **18**:277–290.

[4] Rettger, L. F.
1909. Further studies on fatal septicemia in young chickens, or "white diarrhea." Jour. Med. Res. **21**:115–123.

[5] Rettger, L. F., and F. H. Stoneburn
1909. Bacillary white diarrhea of young chicks. Storrs (Connecticut) Agr. Exp. Sta. Bul. **60**:1–57, figs. 1–7.

[6] ———
1911. Bacillary white diarrhea of young chicks (second report). Storrs (Connecticut) Agr. Exp. Sta. Bul. **68**:279–301, fig. 1–7.

[7] Rettger, L. F., W. F. Kirkpatrick and F. H. Stoneburn
1912. Bacillary white diarrhea of young chicks (third report). Storrs (Connecticut) Agr. Exp. Sta. Bul. **74**:153–185, figs. 1–8.

[8] Rettger, L. F.
1914. Ovarian infection in the domestic fowl and direct transmission of disease to offspring. Jour. Exp. Med. **19**:552–561.

[9] Rettger, L. F., W. F. Kirkpatrick, and R. E. Jones
1914. Bacillary white diarrhea of young chicks (fourth report). Storrs (Connecticut) Agr. Exp. Sta. Bul. **77**:263–309, figs. 1–4.

[10] Jones, F. S.
1910. Fatal septicemia or bacillary white diarrhea in young chickens. New York, State Vet. Col. Ann. Rpt. 1909–10, pp. 111–129.

[11] ———
1911. Further studies on bacillary white diarrhea in young chickens. New York State Vet. Col. Ann. Rpt. 1910–11, pp. 69–88.

[12] Gage, G. E.
1911. Notes on ovarian infection with *Bacterium pullorum* (Rettger) in the domestic fowl. Jour. Med. Res. **24**:491–496.

[13] Jones, F. S.
1913. The value of the macroscopic agglutination test in detecting fowls that are harboring *Bacterium pullorum*. Jour. Med. Res. **27**: 481–495.

[14] Beach, B. A., J. G. Halpin, and C. E. Lampman
1927. Results of white diarrhea investigation. Jour. Amer. Vet. Med. Assn. **70**:597–605.

THE ELIMINATION OF CLOUDY REACTIONS BY THE USE OF FORMALIN AS A PRESERVATIVE FOR *BACTERIUM PULLORUM* ANTIGEN

J. R. BEACH[1] AND S. TER-MICHAELIAN[2]

Introduction

All who have used the agglutination test for the detection of fowls that harbor *Bacterium pullorum* have observed the occurrence of excessive turbidity in many tubes which seriously interfered with accurate reading of the reactions. Hitchner,[3] in 1923, reported that the turbidity resulted from the precipitation of fat that is present in the blood serum of some fowls and that it could be avoided by starving fowls for thirty-six hours before blood samples were drawn. Matthews,[4] in 1926, reported studies which he believed demonstrated that such turbidity was due to the presence of a protein rather than a fatty substance in blood serum of fowls. He stated that this protein substance was soluble in weak alkali solution and that clouding of agglutination tests could be avoided by adding a small amount of sodium hydroxide solution to antigen.

Bushnell, Hinshaw and Payne,[5] in 1926, published a very complete discussion of bacillary white diarrhea in fowls which included a résumé of the methods used by various agricultural experiment station laboratories in making agglutination tests. This résumé shows that in twenty-four of twenty-eight laboratories, phenol is used for preservation of the antigen. The amount of phenol used is 0.5 per cent in nineteen laboratories and 0.4, 0.3, 0.25 and 0.2 per cent phenol in one each of four other laboratories. One laboratory was reported as

[1] Assistant Professor of Veterinary Science and Associate Veterinarian in the Experiment Station.

[2] Research Assistant in Veterinary Science.

[3] Hitchner, E. R. The macroscopic agglutination test as influenced by the fatty content of the blood serum of fowls. Jour. Amer. Vet. Med. Assn. 63: 759–763. 1923.

[4] Matthews, F. P. Obscured reactions in the agglutination test for bacillary white diarrhea. Jour. Immunology 11:499–504. 1926.

[5] Bushnell, L. D., W. R. Hinshaw, and L. F. Payne. Bacillary white diarrhea in fowl. Kansas Agr. Exp. Sta. Tech. Bul. 21:1–858, figs. 1–4. 1926.

using either 0.5 per cent phenol or 0.5 per cent formalin; one as using a coal tar disinfectant (no percentage given); and two as using no preservative in the antigen. It is seen from the above that preservation of antigen by the addition of 0.5 per cent phenol is the prevailing practice. In a further discussion of their own technique and that of other laboratories, these writers disclose that the reason for using 0.3 per cent or a lesser amount of phenol in antigen is to avoid confusing turbidity. The writers also state that "Formolized antigens do not cause a precipitation of the fat-like substance but antigens so prepared are not as reliable as when preserved with phenol." Tittsler, in Pennsylvania, however, is reported by them as using 0.5 per cent formalin for preserving antigen.

The laboratory of the Division of Veterinary Science, California Agricultural Experiment Station, was one of those using antigen containing 0.5 per cent phenol in routine testing of breeding flocks. Difficulty in interpreting the results of the tests on account of turbidity produced by certain sera was frequently encountered. An investigation of means of avoiding turbid or cloudy reactions was, therefore, undertaken. This investigation consisted of a comparison of the results of agglutination tests of the same sera with antigens containing varying amounts of phenol or formalin.

Tests with Antigens Containing 0.1, 0.25, or 0.5 Per Cent Phenol

The antigens were prepared by washing off the growth from 48-hour agar cultures of *Bact. pullorum* with a small amount of physiological salt solution containing 0.5 per cent phenol. For the tests, this was diluted with sufficient physiological saline with or without the addition of phenol to give a reading of 3.5 cm. with a Gates[6] nephelometer and to make the final product contain the desired amount of phenol.

Forty-eight sera were tested with three antigens containing 0.1, 0.25 and 0.5 per cent of phenol respectively. Serum-antigen dilutions of 1–25 and 1–50 were used. Readings were made after 24 hours at 37.5° C and 24 hours at room temperature. The results are as follows:

No positive reactions to the test occurred.

In seventeen of the tests with 0.5 per cent phenolized antigen, there was either increased cloudiness of the fluid or sediment at the bottom of the tubes from the precipitation of a substance from the sera.

[6] Gates, F. L. A method of standardizing bacterial suspensions. Jour. Exp. Med. 31:105–114. 1920.

In ten of the tests with the 0.25 per cent phenolized antigen, there was some increase in cloudiness of the fluid due to a substance in the sera. The cloudiness in these cases was not nearly as marked as that which occurred in the tests of the same sera with 0.5 per cent phenolized antigen.

In none of the tests with the 0.1 per cent phenolized antigen was there any cloudiness or sediment due to precipitation of a substance in the serum. In all 48 tests with this antigen, however, there was either an increase in cloudiness or there was sediment at the bottom of the tubes resulting from the multiplication of contaminating organisms that were present in the sera.

The results of these preliminary agglutination tests of fowl serum with phenolized antigens indicated that cloudiness due to the precipitation of a substance in the serum could be lessened in intensity or entirely avoided by using less than 0.5 per cent phenol in the antigen. When 0.25 per cent phenol was used, the degree of cloudiness from this cause was greatly reduced but not entirely eliminated. When 0.1 per cent phenol was used, no cloudiness from the precipitation of substances in the serum occurred. This latter amount of phenol, however, was insufficient to prevent the multiplication of the contaminating organisms in the serum and, therefore, would be unsatisfactory for use unless the blood was drawn and the test carried out under aseptic conditions.

Tests with Antigens Containing 0.5 Per Cent Phenol, 0.5 Phenol, 0.5 Per Cent Formalin or 0.1 Per Cent Formalin

The 0.5 per cent phenolized antigen and 0.5 per cent formolized antigen were prepared by washing 48-hour agar cultures with a small amount of 0.5 per cent phenolized or formolized physiological salt solution and diluting this concentrated suspension sufficiently with 0.5 per cent phenolized or formolized saline solution at the time of use. The 0.1 per cent formolized antigen was prepared by diluting the concentrated 0.5 per cent formolized antigen with physiological salt solution and 0.1 per cent formolized physiological salt solution at the time of use. The turbidity standard of all antigens was a 3.5 cm. reading with a Gates' nephelometer.

Agglutination tests of 970 sera were made with each of the three antigens. The dilutions of serum and antigen used were 1–25 and 1–50. The results are as follows:

In no case was there any cloudiness or sediment resulting from bacterial multiplication.

Cloudiness occurred in 340, or 35.0 per cent of the tests with phenolized antigen. No cloudiness occurred in the tests with formolized antigens.

A positive reaction in the 0.1 per cent formolized antigen was obtained with 168, or 17.3 per cent of the sera. Of these 168 sera that gave a positive reaction with the 0.1 per cent formolized antigen, 127 sera (or 13.0 per cent of the total sera) also reacted positively with the other two antigens; 13 sera (1.3 per cent of the total sera) also reacted positively with the 0.5 per cent phenolized antigen but not with the 0.5 per cent formolized antigen; 19 sera (1.9 per cent of the total sera) also gave a positive reaction with the 0.5 per cent formolized antigen but not with the phenolized antigen; and 9 sera (0.9 per cent of the total sera) did not react with either of the other two antigens.

All sera that reacted with either the phenolized or 0.5 per cent formolized antigen also reacted with the 0.1 per cent formolized antigen.

Twenty-two of the twenty-eight sera that gave a positive agglutination with the 0.1 per cent formolized antigen and no recognizable agglutination with the phenolized antigen caused clouding of the phenolized antigen. It is possible, therefore, that these twenty-two sera did cause an agglutination of the phenolized antigen which was obscured by the cloudiness. This may account for much of the discrepancy in the results obtained in these tests with the phenolized and 0.1 per cent formolized antigens.

The explanation of the failure of twenty-two of the sera that reacted with the 0.1 per cent formolized antigen to react with the 0.5 per cent formolized antigen, however, is not so apparent. Since the only variable factor was the amount of formalin in the antigens, it seems possible that, in these instances, the 0.5 per cent formalin may have exerted an unfavorable influence on the agglutination of the organisms in the antigen.

The results of these comparative agglutination tests suggested that formalized antigens are more suitable for tests of fowl serum than phenolized antigens. Of the two amounts of formalin used in antigen, i.e., 0.1 per cent and 0.5 per cent, the former seemed more satisfactory. Therefore, additional comparative tests of fowl sera with 0.5 per cent phenolized antigen and 0.1 per cent formolized antigen were carried out.

Tests with Antigens Containing 0.5 Per Cent Phenol or 0.1 Per Cent Formalin

These tests were carried out as opportunity was afforded between February 23 and December 30, 1926, with blood samples from thirty-four flocks.

The methods of preparation and standardization of the antigens were the same as in the preceding tests. Four serum-antigen dilutions, 1–25, 1–50, 1–100, and 1–200, were used in approximately one-third of the tests and two dilutions, 1–25 and 1–50, in the remainder.

Duplicate tests of 4322 sera with two antigens containing 0.5 per cent phenol and 0.1 per cent formalin, respectively, were made. The results are given in table 1.

As shown in table 1, the number of the 4322 sera that reacted with either one or both of the 0.5 per cent phenolized antigen and the 0.1 per cent formolized antigen was 1009 or 23.3 per cent. Of this number, 83 did not react with the phenolized antigen and 41 did not react with the formolized antigen, leaving 885 (20.4 per cent of all tests or 87.7 per cent of all positive tests) that reacted with both antigens.

Cloudiness of the phenolized antigen was caused by 1700 or 39.3 per cent of the sera. The formolized antigen was not affected. The agglutination reaction of 298 of these sera was recorded as positive with both antigens, of 34 as positive with phenolized antigen only, and of 64 as positive with formolized antigen only. By comparing these numbers with the total number of sera that caused agglutination reaction with only one antigen, it is seen that 34 of 41 sera that gave a reaction recorded as positive with phenolized antigen only, and 64 of 83 sera that gave a reaction recorded as positive with formolized antigen only also caused cloudiness of the phenolized antigen.

Since cloudiness of serum-antigen mixtures makes interpretation of agglutination reactions uncertain, it is possible that incorrect readings were made of many or all of the reactions with the phenolized antigen of those sera that caused cloudiness of the phenolized antigen and an agglutination reaction recorded as positive with one antigen only. In such a case, an incorrect interpretation of the agglutination-test reactions with phenolized antigen may have been made of 34 of the 41 sera that were recorded as reacting with phenolized antigen only and of 64 of the 83 sera that were recorded as reacting with formolized antigen only. This would leave but 26 or 0.6 per cent of all tests in which failure to secure the same interpretation of the agglutination reactions with both antigens might not have been due to the real reaction with the phenolized antigen being obscured by cloudiness.

TABLE 1

RESULTS OF AGGLUTINATION TESTS OF 4,322 FOWL SERA WITH 0.5 PER CENT PHENOLIZED ANTIGEN AND 0.1 PER CENT FORMOLIZED ANTIGEN

Number of sera	Number that reacted				Number cloudy with phenolized antigen			
	With both antigens	With phenolized antigen only	With formolized antigen only	Total	Total	Also reacted with both antigens	Also reacted with phenolized antigen only	Also reacted with formolized antigen only
200	3	0	5	8	151	0	0	4
65	0	0	0	0	10	0	0	0
56	3	0	0	3	46	0	0	0
92	10	0	1	11	27	1	0	0
42	1	0	0	1	25	1	0	0
11	0	0	0	0	0	0	0	0
9	1	0	0	1	1	0	0	0
9	2	0	0	2	0	0	0	0
171	54	0	2	56	52	10	0	2
54	9	0	0	9	3	0	0	0
176	34	0	10	44	61	9	0	6
170	27	0	16	43	87	12	0	14
215	0	0	1	1	56	0	0	1
218	38	0	0	38	49	2	0	0
164	24	0	5	29	86	6	0	5
125	43	0	4	47	11	4	0	4
203	21	0	0	21	103	1	0	0
190	48	1	3	52	63	1	0	2
161	34	0	2	36	68	5	0	1
70	0	0	0	0	22	0	0	0
114	27	0	0	27	21	0	0	0
199	67	0	0	67	64	2	0	0
50	23	0	0	23	7	0	0	0
134	13	0	2	15	48	4	0	0
96	3	0	0	3	62	0	0	0
131	2	0	0	2	82	0	0	0
13	2	0	0	2	0	0	0	0
33	5	0	0	5	0	0	0	0
21	4	0	0	4	12	0	0	0
6	0	0	0	0	0	0	0	0
36	6	0	0	6	10	0	0	0
36	6	0	0	6	0	0	0	0
156	29	0	1	30	31	6	0	1
313	167	30	10	207	275	164	28	8
152	21	2	0	23	16	0	0	0
151	21	0	4	25	1	0	0	1
280	137	8	17	162	150	70	6	15
4322	885	41	83	1009	1700	298	34	64

Seven of the fowls whose blood serum had agglutinated the formolized antigen and had neither clouded nor agglutinated the phenolized antigen were secured for autopsy. The postmortem and bacteriological examinations of two of these birds were negative. Four birds had abnormal yolks in the ovaries. *Bact. pullorum* was isolated from three of these. The seventh bird exhibited no ovarian abnormalities, but a mass of fibrinous exudate was present in the pericardial sac. *Bact. pullorum* was isolated from the exudate. These results demonstrate that at least a part of the fowls that reacted with the formolized antigen only were carriers of *Bact. pullorum.*

Variations in the Degree of the Reactions with the Two Antigens

The preceding discussion of the comparative results of the agglutination tests with 0.5 per cent phenolized and 0.1 per cent formolized antigens has shown that 885 or 20.4 per cent of the sera gave a positive reaction with both antigens. In these tests, partial or complete agglutination in any serum-antigen dilution was considered a positive reaction. This classification, therefore, serves to differentiate the sera which produced no agglutination of either one or both antigens from those which produced some agglutination in one or more dilutions with each antigen, but does not indicate whether agglutination occurred in one or more dilutions or whether the number of dilutions in which agglutination occurred was the same for both antigens. In making an accurate comparison of the results with the two antigens, however, consideration of the degree of agglutination obtained with each antigen must be given. A summary of the data on this point is, therefore, included in this paper.

Two dilutions, i.e., 1–25 and 1–50, were used in 3021 tests and four dilutions, i.e., 1–25, 1–50, 1–100 and 1–200 in 1301 tests. The results are as follows:

Two-dilution Tests.—A positive reaction with both antigens was obtained in 641 tests. In 546, or 85.1 per cent, agglutination occurred in the same dilutions of both antigens as follows:

35 sera agglutinated the 1–25 dilution only.
511 sera agglutinated both the 1–25 and 1–50 dilutions.

In 95, or 14.8 per cent, of the positive tests, agglutination did not occur in the same dilutions of both antigens. The variations in these agglutination reactions were:

40 sera agglutinated the 1–25 dilution only of phenolized antigen and both dilutions of formolized antigen.
55 sera agglutinated both dilutions of phenolized antigen and the 1–25 dilution only of formolized antigen.

There was cloudiness of the phenolized antigen in 57 of the 95 tests. This might have been responsible for much of the difference in the readings of the reactions with the two antigens in these tests.

Four-dilution Tests.—A positive reaction in both antigens was obtained in 244 tests.

In 126, or 51.6 per cent, agglutination occurred in the same dilutions of both antigens as follows:

27 sera agglutinated the 1–25 dilution only.
39 sera agglutinated the 1–25 and 1–50 dilutions only.
8 sera agglutinated the 1–25, 1–50 and 1–100 dilutions.
59 sera agglutinated the 1–25, 1–50, 1–100 and 1–200 dilutions.

In 118, or 48.3 per cent, of the positive tests, the dilutions in which agglutination occurred were not the same for both antigens. The agglutination titre in fourteen of these tests was higher with the phenolized than with the formolized antigen, and in 104 tests the titre was higher with the formolized than with the phenolized antigen. The variations in these agglutination reactions are given in table 2.

TABLE 2

VARIATION IN THE DILUTIONS OF PHENOLIZED AND FORMOLIZED ANTIGENS AGGLUTINATED BY THE SAME SERA. DILUTIONS OF 1–25, 1–50, 1–100 AND 1–200 WERE USED

Number of sera	Dilutions of phenolized antigen agglutinated	Dilutions of formolized antigen agglutinated	Number of sera causing clouding of phenolized antigen
2	1–25, 1–50, 1–100, 1–200	1–25, 1–50	0
3	1–25, 1–50, 1–100, 1–200	1–25, 1–50, 1–100	1
4	1–25, 1–50, 1–100	1–25, 1–50	1
5	1–25, 1–50	1–25	1
6	1–25	1–25, 1–50, 1–100, 1–200	3
28	1–25, 1–50	1–25, 1–50, 1–100, 1–200	6
28	1–25, 1–50, 1–100	1–25, 1–50, 1–100, 1–200	3
14	1–25, 1–50	1–25, 1–50, 1–100	3
5	1–25	1–25, 1–50, 1–100	0
23	1–25	1–25, 1–50	9

It can be seen from the preceding data that there was little difference in the agglutination of the phenolized and formolized antigens in the 1–25 and 1–50 dilutions. Much of the difference that did exist might have been due to incorrect reading of the reaction in the phenolized antigen because of clouding of that antigen by some of the sera. A considerably larger number of sera, however, caused agglutination of the 1–100 and 1-200 dilutions of formolized antigen than in the corresponding dilutions of phenolized antigen.

Discussion

The results of the 4322 comparative tests indicate that antigen containing 0.1 per cent formalin is satisfactory for making agglutination tests of blood serum from fowls. There was little difference in either the number or distribution of the sera which reacted with the two antigens. In the tests in which four dilutions were used and in which reactions to both antigens in at least one dilution were obtained, more sera caused agglutination in the 1–100 and 1–200 dilutions of formolized antigen than in the corresponding dilutions of phenolized antigen.

The cloudiness which occurred in 1700 tests with phenolized antigen did not appear in the corresponding tests with formolized antigen. In this respect, the formolized antigen was more satisfactory than the phenolized antigen.

It was observed that the clumps of bacteria formed by the agglutination of the organisms in the formolized antigen were smaller and more easily broken up than the clumps of bacteria in the phenolized antigen. This was of no importance when complete agglutination occurred, but did make the reading of partial agglutinations more difficult in the formolized antigen than in the phenolized antigen. This feature of the behavior of formolized antigen, however, is an unimportant source of error in the interpretation of agglutination reactions when compared with the frequently-occurring cloudiness of phenolized antigen.

PUBLICATIONS AVAILABLE FOR FREE DISTRIBUTION

BULLETINS

No.

253. Irrigation and Soil Conditions in the Sierra Nevada Foothills, California.
261. Melaxuma of the Walnut, "Juglans regia."
262. Citrus Diseases of Florida and Cuba Compared with Those of California.
263. Size Grades for Ripe Olives.
268. Growing and Grafting Olive Seedlings.
273. Preliminary Report on Kearney Vineyard Experimental Drain.
275. The Cultivation of Belladonna in California.
276. The Pomegranate.
277. Sudan Grass.
278. Grain Sorghums.
279. Irrigation of Rice in California.
283. The Olive Insects of California.
294. Bean Culture in California.
304. A Study of the Effects of Freezes on Citrus in California.
310. Plum Pollination.
312. Mariout Barley.
313. Pruning Young Deciduous Fruit Trees.
319. Caprifigs and Caprification.
324. Storage of Perishable Fruit at Freezing Temperatures.
325. Rice Irrigation Measurements and Experiments in Sacramento Valley, 1914–1919.
328. Prune Growing in California.
331. Phylloxera-Resistant Stocks.
335. Cocoanut Meal as a Feed for Dairy Cows and Other Livestock.
339. The Relative Cost of Making Logs from Small and Large Timber.
340. Control of the Pocket Gopher in California.
343. Cheese Pests and Their Control.
344. Cold Storage as an Aid to the Marketing of Plums.
346. Almond Pollination.
347. The Control of Red Spiders in Deciduous Orchards.
348. Pruning Young Olive Trees.
349. A Study of Sidedraft and Tractor Hitches.
350. Agriculture in Cut-over Redwood Lands.
352. Further Experiments in Plum Pollination.
353. Bovine Infectious Abortion.
354. Results of Rice Experiments in 1922.
357. A Self-mixing Dusting Machine for Applying Dry Insecticides and Fungicides.
358. Black Measles, Water Berries, and Related Vine Troubles.
361. Preliminary Yield Tables for Second Growth Redwood.
362. Dust and the Tractor Engine.
363. The Pruning of Citrus Trees in California.
364. Fungicidal Dusts for the Control of Bunt.
365. Avocado Culture in California.
366. Turkish Tobacco Culture, Curing and Marketing.
367. Methods of Harvesting and Irrigation in Relation of Mouldy Walnuts.
368. Bacterial Decomposition of Olives during Pickling.
369. Comparison of Woods for Butter Boxes.

No.

370. Browning of Yellow Newtown Apples.
371. The Relative Cost of Yarding Small and Large Timber.
372. The Cost of Producing Market Milk and Butterfat on 246 California Dairies.
373. Pear Pollination.
374. A Survey of Orchard Practices in the Citrus Industry of Southern California.
375. Results of Rice Experiments at Cortena, 1923.
376. Sun-Drying and Dehydration of Walnuts.
377. The Cold Storage of Pears.
379. Walnut Culture in California.
380. Growth of Eucalyptus in California Plantations.
381. Growing and Handling Asparagus Crowns.
382. Pumping for Drainage in the San Joaquin Valley, California.
383. Monilia Blossom Blight (Brown Rot) of Apricot.
385. Pollination of the Sweet Cherry.
386. Pruning Bearing Deciduous Fruit Trees.
387. Fig Smut.
388. The Principles and Practice of Sun-drying Fruit.
389. Berseem or Egyptian Clover.
390. Harvesting and Packing Grapes in California.
391. Machines for Coating Seed Wheat with Copper Carbonate Dust.
392. Fruit Juice Concentrates.
393. Crop Sequences at Davis.
394. Cereal Hay Production in California. Feeding Trials with Cereal Hay.
395. Bark Diseases of Citrus Trees.
396. The Mat Bean (Phaseolus aconitifolius).
397. Manufacture of Roquefort Type Cheese from Goat's Milk.
398. Orchard Heating in California.
399. The Blackberry Mite, the Cause of Redberry Disease of the Himalaya Blackberry, and its Control.
400. The Utilization of Surplus Plums.
401. Cost of Work Horses on California Farms.
402. The Codling Moth in Walnuts.
403. Farm-Accounting Associations.
404. The Dehydration of Prunes.
405. Citrus Culture in Central California.
406. Stationary Spray Plants in California.
407. Yield, Stand and Volume Tables for White Fir in the California Pine Region.
408. Alternaria Rot of Lemons.
409. The Digestibility of Certain Fruit By-products as Determined for Ruminants.
410. Factors Affecting the Quality of Fresh Asparagus after it is Harvested.
411. Paradichlorobenzene as a Soil Fumigant.
412. A Study of the Relative Values of Certain Root Crops and Salmon Oil as Sources of Vitamin A for Poultry.
413. The California Poultry Industry; a Statistical Study.
414. Planting and Thinning Distances for Deciduous Fruit Trees.

No.
87. Alfalfa.
117. The Selection and Cost of a Small Pumping Plant.
127. House Fumigation.
129. The Control of Citrus Insects.
136. *Melilotus indica* as a Green-Manure Crop for California.
144. Oidium or Powdery Mildew of the Vine.
157. Control of the Pear Scab.
160. Lettuce Growing in California.
164. Small Fruit Culture in California.
166. The County Farm Bureau.
170. Fertilizing California Soils for the 1918 Crop.
173. The Construction of the Wood-Hoop Silo.
178. The Packing of Apples in California.
179. Factors of Importance in Producing Milk of Low Bacterial Count.
190. Agriculture Clubs in California.
199. Onion Growing in California.
202. County Organizations for Rural Fire Control.
203. Peat as a Manure Substitute.
209. The Function of the Farm Bureau.
210. Suggestions to the Settler in California.
212. Salvaging Rain-Damaged Prunes.
215. Feeding Dairy Cows in California.
217. Methods for Marketing Vegetables in California.
220. Unfermented Fruit Juices.
228. Vineyard Irrigation in Arid Climates.
230. Testing Milk, Cream, and Skim Milk for Butterfat.
231. The Home Vineyard.
232. Harvesting and Handling California Cherries for Eastern Shipment.
234. Winter Injury to Young Walnut Trees during 1921–22.
235. Soil Analysis and Soil and Plant Inter-relations.
236. The Common Hawks and Owls of California from the Standpoint of the Rancher.
237. Directions for the Tanning and Dressing of Furs.
238. The Apricot in California.
239. Harvesting and Handling Apricots and Plums for Eastern Shipment.
240. Harvesting and Handling Pears for Eastern Shipment.
241. Harvesting and Handling Peaches for Eastern Shipment.
243. Marmalade Juice and Jelly Juice from Citrus Fruits.
244 Central Wire Bracing for Fruit Trees.
245. Vine Pruning Systems.
247. Colonization and Rural Development.
248. Some Common Errors in Vine Pruning and Their Remedies.
249. Replacing Missing Vines.
250. Measurement of Irrigation Water on the Farm.
252. Supports for Vines.
253. Vineyard Plans.
254. The Use of Artificial Light to Increase Winter Egg Production.

No.
255. Leguminous Plants as Organic Fertilizer in California Agriculture.
256. The Control of Wild Morning Glory.
257. The Small-Seeded Horse Bean.
258. Thinning Deciduous Fruits.
259. Pear By-products.
261. Sewing Grain Sacks.
262. Cabbage Growing in California.
263. Tomato Production in California.
264. Preliminary Essentials to Bovine Tuberculosis Control.
265. Plant Disease and Pest Control.
266. Analyzing the Citrus Orchard by Means of Simple Tree Records.
267. The Tendency of Tractors to Rise in Front: Causes and Remedies.
269. An Orchard Brush Burner.
270. A Farm Septic Tank.
272. California Farm Tenancy and Methods of Leasing.
273. Saving the Gophered Citrus Tree.
274. Fusarium Wilt of Tomato and its Control by Means of Resistant Varieties.
276. Home Canning.
277. Head, Cane, and Cordon Pruning of Vines.
278. Olive Pickling in Mediterranean Countries.
279. The Preparation and Refining of Olive Oil in Southern Europe.
281. The Results of a Survey to Determine the Cost of Producing Beef in California.
282. Prevention of Insect Attack on Stored Grain.
283. Fertilizing Citrus Trees in California.
284. The Almond in California.
285. Sweet Potato Production in California.
286. Milk Houses for California Dairies.
287. Potato Production in California.
288. Phylloxera Resistant Vineyards.
289. Oak Fungus in Orchard Trees.
290. The Tangier Pea.
291. Blackhead and Other Causes of Loss of Turkeys in California.
292. Alkali Soils.
293. The Basis of Grape Standardization.
294. Propagation of Deciduous Fruits.
295. The Growing and Handling of Head Lettuce in California.
296. Control of the California Ground Squirrel.
298. The Possibilities and Limitations of Coöperative Marketing.
299. Poultry Breeding Records.
300. Coccidiosis of Chickens.
301. Buckeye Poisoning of the Honey Bee.
302. The Sugar Beet in California.
303. A Promising Remedy for Black Measles of the Vine.
304. Drainage on the Farm.
305. Liming the Soil.
306. A General Purpose Soil Auger and its Use on the Farm.
307. American Foulbrood and its Control.
308. Cantaloupe Production in California.

The publications listed above may be had by addressing

College of Agriculture,
University of California,
Berkeley, California.